# DAIDS

# VIROLOGY MANUAL

# FOR HIV LABORATORIES

**Version**
**January 1997**

**Compiled by**

**THE DIVISION OF AIDS**

**NATIONAL INSTITUTE OF ALLERGY & INFECTIOUS DISEASES**

**NATIONAL INSTITUTES OF HEALTH**

**and**

**COLLABORATING INVESTIGATORS**

# NASBA™ HIV-1 RNA QT QUANTITATIVE RNA ASSAY

## I. PRINCIPLE

Acquired Immunodeficiency Syndrome (AIDS) is an immunosuppressive disorder characterized by depletion of the CD4+ T cell population. A progressive, severe immunodeficient state is accompanied by a broad variety of clinical manifestations including opportunistic infections, an array of malignancies, and the frequent presence of neurological disorders.

The etiologic agent of AIDS is the Human Immunodeficiency Virus (HIV). It is transmitted by sexual contact, through contaminated injection needles, or through administration of contaminated blood or blood products. HIV is also capable of passing through the placenta. So far, two types of HIV have been found to cause AIDS: HIV-1, first isolated in 1983, and HIV-2, a second distinct but related type, first isolated in 1985.

The conventional method for detection of HIV infection is through serologic identification of an immunologic response to HIV, by means of enzyme-linked immunosorbent assays (ELISA), and confirmation of the results with more specific assays (western blot).

Unlike these indirect methods, nucleic acid amplification techniques such as Reverse Transcriptase PCR and NASBA™ do not depend on the development of an immunologic response to HIV, which occasionally takes six months or more from the time of infection to occur. They directly test for the presence of HIV RNA and thus can detect HIV infection before seroconversion. In addition, they are more sensitive than p24 antigen assays. The advantage of NASBA™ over Reverse Transcriptase PCR is that it requires no separate reverse transcriptase step or, as amplification is isothermal, any thermocycler equipment.

Nucleic acid amplification is also suitable for the quantitation of HIV-1 RNA in plasma and serum samples. Determination of the viral load appears to be a valuable marker for the prediction of disease progression and for monitoring the efficacy of anti-viral therapy especially in the early stage of the disease when conventional markers are often negative.

Quantitation with NASBA™ HIV-1 RNA QT is based on co-amplification of HIV-1 sample RNA together with internal calibrators, a technique which has proved to be superior to other quantitation methods. The quantity of amplified RNA is measured by means of electrochemiluminescence (ECL).

The NASBA™ HIV-1 RNA QT assay comprises four separate stages: Nucleic acid release, Nucleic acid isolation, Nucleic acid amplification, and Nucleic acid detection.

The nucleic acid release step is accomplished by adding the sample to lysis buffer containing guanidine thiocyanate and Triton X-100. Any viral particles, RNases, and DNases present in the sample are disintegrated and nucleic acid is released.

The next step is nucleic acid isolation. Three synthetic RNAs (Qa,Qb,Qc) of known high, medium, and low concentration, respectively, are added to the lysis buffer containing the released nucleic acid. These RNAs serve as internal calibrators, each differing from the HIV-1 wild type (WT) RNA by only a small sequence. Under high salt conditions, all nucleic acid in the buffer, including the calibrators, is bound to silicon dioxide particles. These particles, acting as the solid phase, are washed several times. Finally, the nucleic acid is eluted from the solid phase.

Nucleic acid amplification follows the nucleic acid isolation step. Any wild type HIV-1 RNA present in the eluted nucleic acid is co-amplified with three internal calibrators. Amplification is based on primer extension: the wild type RNA and the calibrator RNAs serve as templates for the extension of gag region primer 1 (containing the T7-RNA polymerase recognition site) by Avian Myeloblastosis Virus reverse transcriptase (AMV-RT). Extension is followed by degradation of the template RNAs by RNase H, synthesis of the second DNA strand through extension of primer 2 by AMV-RT, and RNA synthesis by T7-RNA polymerase. With RNA synthesis the system enters the isothermal cyclic phase, resulting in the accumulation of wild-type and calibrator RNA amplificates.

The final step is nucleic acid detection. Detection of HIV-1 RNA in a sample is based on the NASBA™ QR System electrochemiluminescence principle. To separate the amplificates (WT,Qa,Qb, and Qc), aliquots of the amplified sample are added to four hybridization solutions, each specific for one of the amplificates. Here, the respective amplificates are hybridized with a bead-oligo (i.e., a biotin -oligo bound to streptavidin coated magnetic beads acting as the solid phase) and a ruthenium-labeled probe. The magnetic beads carrying the hybridized amplificate/probe complex are captured on the surface of an electrode by means of a magnet. Voltage applied to this electrode triggers the electrochemiluminescence (ECL) reaction. The light emitted by the hybridized ruthenium labeled probes is proportional to the amount of amplificate. Calculation based on the relative amounts of the sample amplificates reveals the original amount of wild-type HIV-1 RNA in the sample.

## II. SPECIMEN REQUIREMENTS

Plasma is the specimen of choice, although serum may be used. No special preparation or fasting of the patient is necessary.

1. Transfer greater than 2 mL of blood into an EDTA, citrate or heparin vacutainer.

2. Prepare plasma as soon as possible, i.e., preferably within two hours after collection, by centrifuging the capped tube for 15 $\pm$ 1 minutes at 1000 g.

3. Do not uncap tubes in the presence of other open tubes containing patient material.

4. Add specimens within two hours after collection to the lysis buffer tubes to avoid HIV-1 RNA degradation, or store immediately at -70$^{o}$C as a back-up. In lysis buffer, the HIV-1 RNA can be stored at room temperature (15-30$^{o}$C) for a

maximum of one day; for a maximum of one week at 2-8$^{o}$C; long-term storage is possible at -20$^{o}$C or, preferably, at -70$^{o}$C.

Note: The following should be considered unacceptable:

Whole unseparated blood.
Grossly lipemic or hemolyzed samples.
Specimens repeatedly frozen and thawed or those containing particulate matter may give erroneous results.

## III REAGENTS

A. Precautions

Alterations in the physical appearance of the test kit material may indicate instability or deterioration. Expiration dates shown on component labels indicate the date beyond which components should not be used. Prepare reagents before starting nucleic acid release, isolation, amplification and detection, respectively.

**AVOID CONTAMINATION: STORE AND PREPARE REAGENTS FOR NUCLEIC ACID RELEASE, ISOLATION, AMPLIFICATION AND DETECTION AT THE LABORATORY AREAS WHERE NUCLEIC ACID RELEASE, ISOLATION, AMPLIFICATION AND DETECTION, RESPECTIVELY, ARE TO BE PERFORMED**.

Make sure reagents and samples are at room temperature (15-30$^{o}$C) before starting nucleic acid release, isolation, amplification and detection, respectively.

Before opening a tube which contains lyophilized material , make certain that it is at the bottom of the tube.

Use prepared reagents immediately. Storage of prepared or opened reagents is not recommended.

CAUTION: The lysis buffer and the wash buffer contain guanidine thiocyanate. Guanidine thiocyanate is harmful by inhalation, in contact with skin and if swallowed. Contact with acids liberates very toxic gas. The elution buffer, the enzyme diluent and the detection diluent contain sodium azide. When discarding into sewerage, always flush with copious quantities of water. This helps prevent formation of metallic azides which, when highly concentrated in metal plumbing, may be potentially explosive. Plumbing should be periodically decontaminated according to appropriate guidelines.

B. The NASBA™ HIV-1 RNA QT provides the following reagents.

1. The regents needed to perform 50 NASBA™ HIV-1 RNA QT tests are supplied in three separate boxes containing: lysis buffer, reagents for nucleic acid isolation and amplification, and reagents for nucleic acid detection.

   a. Nucleic Acid Release Reagent

      1) Lysis Buffer: 50 tubes (0.9 mL/tube) 5 mol/L guanidine thiocyanate, Triton X-100, Tris/HCl. Store at 2 -8$^0$C. Note manufacturer's outdate.

   b. Nucleic Acid Isolation

      1) Wash Buffer: 5 vials (22 mL/vial) 5 mol/L guanidine thiocyanate, Tris/HCl. Store at 2 -8$^0$C. Note manufacturer's outdate.

      2) Silica: 5 tubes (0.8 mL/tube). Store at 2 - 8$^0$C. Note manufacturer's outdate.

      3) Elution Buffer: 5 tubes (1.5 mL/tube) Tris/HCl Preservative: 0.9 g/L sodium azide. Color Code: White. Store at 2 -8$^0$C. Note manufacturer's outdate.

      4) Calibrator: 5 foiled-packed tubes (6.5 mg/tube) lyophilized synthetic RNA (Qa, QB, and Qc) sphere; each tube contained in a foil pack with silica gel desiccant. Color Code: Yellow. Store at 2 -8$^0$C. Note manufacturer's outdate.

   c. Nucleic Acid Amplification Reagents

      1) Enzymes: 5 foil-packed tubes (20 mg/tube) lyophilized AMV-RT (chicken), RNase H T7-RNA polymerase, BSA and nucleotides; each contained in a foil pack with silica gel desiccant. Color Code: Red. Store at 2 -8$^0$C. Note manufacturer's outdate.

      2) Enzyme Diluent: 5 tubes (0.2 mL/tube) Tris/HCl Preservative: 0.9 g/L sodium azide. Color Code: Red. Store at 2 -8$^0$C. Note manufacturer's outdate.

      3) Primers: 5 foil-packed tubes (0.5 mg/tube) lyophilized sphere with synthetic primers, dithiothreitol, KCl and $MgCl_2$; each tube contained in a foil pack with silica gel. Color Code: Blue. Store at 2 -8$^0$C. Note manufacturer's outdate.

      4) Primer Diluent: 5 tubes (0.2 mL/tube) Tris/HCl, 30% DMSO Color Code: Blue. Store at 2 - 8$^0$C. Note manufacturer's outdate.

d. Nucleic Acid Detection

1) Bead-oligo: 2 tubes (1.68 mL/tube) biotin-oligo bound to streptavidin-coated magnetic beads. Preservative: 1 g/L 2-chloro-acetamide. Color Code: Lilac. Store at 2 -8$^0$C. Note manufacturer's outdate.

2) WT Probe: 1 tube (0.84 mL) Ruthenium-labeled oligo. Preservative: 5g/L 2-chloro-acetamide. Color Code: White. Store at 2 -8$^0$C. Note manufacturer's outdate.

3) Qa Probe: 1 tube (0.84 mL) Ruthenium-labeled oligo. Preservative: 5 g/L 2-chloro-acetamide. Color Code: Red. Store at 2 -8$^0$C. Note manufacturer's outdate.

4) Qb Probe: 1 tube (0.84 mL) Ruthenium-labeled oligo. Preservative: 5 g/L 2-chloro-acetamide. Color Code: Yellow. Store at 2 -8$^0$C. Note manufacturer's outdate.

5) Qc Probe: 1 tube (0.84 mL) Ruthenium-labeled oligo. Preservative: 5g/L 2-chloro-acetamide. Color Code: Blue. Store at 2 -8$^0$C. Note manufacturer's outdate.

6) Detection Diluent: 1 vial (8.4 mL) Tris/HCl. Preservative: 0.9 g/L sodium azide. Store at 2 - 8$^0$C. Note manufacturer's outdate.

7) Instrument Reference Solution: Streptavidin- coated magnetic beads. Store at 2 - 8$^0$C. Note manufacturer's outdate.

B. Additional reagents required but not provided are:

1. 70% Ethanol (prepared from 95% ethanol, ACS quality); prepare tubes of 12 mL each and store at room temperature (15-30$^O$C).

2. Acetone (analytical grade); prepare tubes of 12 mL each and store at room temperature (15-30$^O$C).

## IV. EQUIPMENT AND SUPPLIES

A. Nucleic Acid Release

EDTA, citrate, or heparin vacutainer tubes.
Centrifuge suitable for vacutainer tubes.
Tubes for plasma or serum storage (1-5 mL).
Disposable transfer pipettes (5 mL).

Absorbent tissue.

B. Nucleic Acid Isolation

RNase free 1.5 mL test tubes (preferably screw-cap), which can be centrifuged at 10,000 g, and 1.5 mL test tube racks.
Calibrated disposable pipette tips (10, 100, (2X) 1000 µL) with aerosol resistant tips.
Heating block (or water bath) capable of heating 1.5 mL test tubes to 56 $\pm$ 1°C.
Timer.
Vortex.
Table top centrifuge for 1.5 mL test tubes (capable of 10,000 g.)

C. Nucleic Acid Amplification

Calibrated disposable tip pipettes (10 and 100 µL) with aerosol resistant tips.
Two heating blocks (or water baths) capable of heating 1.5 mL test tubes to 41 $\pm$ 0.5°C and 65 $\pm$ 1° C.
Two calibrated mercury thermometers (precision $\pm$ 0.2°C).
Water bath capable of heating 1.5 mL test tubes to 41 $\pm$ 0.5°C
Timer.
Vortex.
Table top centrifuge for 1.5 mL test tubes (capable of 10,000 g).
1.5 mL test tube racks.

D. Nucleic Acid Detection

Calibrated disposable tip pipettes for variable volumes (5 to 1000 µL) with corresponding tips.
Heating block (or water bath) capable of heating 1.5 mL test tubes to 41 $\pm$ 0.5°C.
Timer.
Vortex.
Table top centrifuge for 1.5 mL test tubes (capable of 10,000 g).
RNase free 1.5 mL test tubes (preferably screw-cap), which can be centrifuged at 10,000 g, and 1.5 mL test tube racks.
NASBA™ QR System with PC, Assay Buffer and Cleaning Solution.
Polypropylene tubes (5 mL) for hybridization (250 tubes per kit).
Repeating pipette with disposable tips (0.500 mL and 5.00 mL).
Vials (50 mL) with screw cap for disposal of pipette tips.
Shaker capable of 1100 rpm.
Adhesive tape.

## V. PROCEDURE

A. Nucleic Acid Release

1. Preparation of Lysis Buffer

a. Prewarm lysis buffer tubes for about 30 minutes (suggested temperature: 37$^{O}$C) before starting the assay to make sure that any crystals in the lysis buffer have dissolved.

b. Protect the lysis buffer from excessive light.

2. Nucleic Acid Release Procedure

a. Spin lysis buffer tubes (i.e., centrifuge the lysis buffer tubes at a speed and time required to spin all fluid down to the bottom of the tubes e.g., 15 seconds at 10,000 g).

b. Uncap tube containing prepared specimen (refer to section II.).

c. Transfer 100 µL of the prepared specimen into a lysis buffer tube. NOTE: smaller specimen volumes from 10-100 µL may also be used. Note that use of smaller specimen volumes has effect on the interpretation of results.

d. Store residual specimen in a separate storage tube (for possible use in other serological tests) at -20$^{O}$C or preferably -70$^{O}$C.

e. Close tubes.

f. Vortex lysis buffer.

g. Prepare next sample in the same way.

Note: The lysis buffer tubes with the sample can be stored at room temperature for 24 hrs, 7 days at 2-8$^{0}$C, and long term storage at -70$^{0}$C.

B. Nucleic Acid Isolation

1. Preparation of Nucleic Acid Isolation Reagents

a. Prewarm wash buffer tubes for about 30 minutes (suggested temperature: 37$^{O}$C) before starting the assay to make sure that any crystals in the wash buffer have dissolved.

b. Protect the wash buffer from excessive light.

c. Vortex the silica tube before starting the isolation procedure until an opaque suspension is formed. The silica particles will settle again loosely. Vortex again before each pipetting step.

d. Reconstitute the calibrator (yellow) in 220 µL elution buffer (white).

e. Vortex the solution.

Note: Use within one hour after preparation. Number of calibrator molecules per 20 µL: Qa $10^6$, Qb $10^5$, Qc $10^4$.

2. Nucleic Acid Isolation Procedure

a. Spin lysis buffer tubes.

b. To each lysis buffer tube add 20 µL calibrator solution, vortex, and spin the lysis buffer tubes.

c. To each tube add 50 µL of vortexed silica suspension.

d. Leave the tubes for 10 $\pm$ 1 minutes at room temperature (15-30$^{o}$C). NOTE: VORTEX LYSIS BUFFER TUBES REGULARLY, E.G., EVERY TWO MINUTES, TO PREVENT SILICA FROM SETTLING ON THE BOTTOM.

e. Spin lysis buffer tubes.

f. Remove the supernatant from the lysis buffer tubes with a 1000 µL disposable tip pipette, using a fresh sterile tip for each tube; avoid whirling up the pellet.

g. Wash the silica pellet in the lysis buffer tubes five times i.e., :

1) twice (2X) with wash buffer
2) twice (2X) with 70% ethanol
3) once (1X) with acetone

h. Wash Procedure:

1) Add 1 mL of wash buffer/ethanol/acetone to each lysis buffer tube with a **second** 1000 µL disposable tip pipette, using a fresh sterile tip for each lysis buffer tube.

2) Vortex until pellets are resuspended.

3) Spin lysis buffer tubes.

4) Remove the supernatant with a 1000 µL tip pipette, using a fresh sterile tip for each lysis buffer tube.

5) Use a clean tube rack for next wash.

i. Dry the silica pellets in open lysis buffer tubes at 56$^{o}$C for 10 $\pm$ 1 minutes in a heating block.

NOTE: COVER THE LYSIS BUFFER TUBES WITH TISSUE TO AVOID CONTAMINATION.

j. Check if lysis buffer tubes are dry (the silica pellet will be white). If tubes are dry add 50 µL elution buffer to each lysis buffer tube and resuspend the silica pellets by vortexing.

k. Leave the resuspended silica for 10 $\pm$ 1 minutes at 56$^{o}$C to elute the nucleic acid. NOTE: VORTEX EVERY 2 MINUTES DURING ELUTION.

l. Centrifuge lysis buffer tubes 2 minutes at 10,000g.

m. Transfer 5 µL of the supernatant nucleic acid from the lysis buffer tube to a fresh test tube.

n. Store remaining 45 µL supernatant with silica pellet as a back-up at -20$^{o}$C or, preferably, at -70$^{o}$C.

o. Transfer the test tubes containing the 5 µL supernatant nucleic acid to the amplification lab area.

p. If the test tubes are not to be immediately used for amplification, store at -20$^{o}$C or, preferably, at -70$^{o}$C.

C. Amplification

1. Preparation of Amplification Reagents

a. Add 45 µL enzyme diluent (red) to the lyophilized enzymes (red).

b. Leave for 15 minutes; ensure complete reconstitution by gently "rolling" the closed tube every 5 minutes between your fingers.

NOTE: USE WITHIN 1 HOUR AFTER PREPARATION. DO NOT VORTEX.

c. Add 120 µL primer diluent (blue) to the lyophilized primers (blue) and vortex. NOTE: USE WITHIN 1 HOUR AFTER PREPARATION.

2. Amplification Procedure

NOTE: AMPLIFICATION STEP a. TO d. MUST BE PERFORMED IN A FUME HOOD TO REDUCE THE RISK OF CONTAMINATION; STEP f. and g. SHOULD PREFERABLY ALSO BE PERFORMED IN A FUME HOOD.

a. Add 10 µL of primer solution to each test tube containing 5 µL supernatant nucleic acid and then close the tube.

b. Incubate test tubes for 5 $\pm$ 1 minutes at 65 $\pm$ 1$^{o}$C.

c. Cool test tubes for a minimum of 5 minutes at 41 $\pm$ 0.5$^{o}$C.

d. Mix enzyme solution by "rolling" the closed tube between your fingers. Add 5 µL of enzyme to each test tube. Mix gently by tapping the test tube and incubate tubes for at least 5 minutes at 41 $\pm$ 0.5$^{o}$C.

NOTE: AVOID ANY UNNECESSARY DELAY BETWEEN THIS INCUBATION AND INCUBATION IN STEP f. BELOW TO PREVENT DISCONTINUATION OF THE AMPLIFICATION PROCESS.

e. Transfer tubes to the detection area and spin the test tubes.

f. Incubate the test tubes at 41 $\pm$ 0.5$^{o}$C for 90 $\pm$ 5 minutes, using a water bath.

g. Store test tubes that are not to be immediately used for detection up to 1 month at -20$^{o}$C.

D. Detection

1. Preparation of Detection Reagents

a. For Hybridization solution 1, vortex bead-oligo (lilac) until an opaque solution is formed. Immediately after vortexing, add 130 µL bead-oligo to a fresh tube and add 130 µL WT probe (white).

b. For hybridization solutions 2 to 4 follow the same procedure, replacing the WT probe by Qa (red), Qb (yellow) and Qc (blue) probe, respectively.

c. Vortex hybridization solutions before use.
d. Add 1.3 mL detection diluent to a fresh test tube.

2. Detection Procedure

**NOTE: DETECTION SHOULD PREFERABLY BE PERFORMED IN A FUME HOOD TO REDUCE THE RISK OF CONTAMINATION**.

a. For each amplified sample, place four fresh 5 mL polypropylene tubes ready for use in a rack (referred to as hybridization tubes 1 to 4 in the following). Place one additional fresh 5 mL polypropylene tube ready for use as a blank (i.e., detection diluent instead of amplified sample is added to this tube).

b. Vortex hybridization solutions until an opaque solution is formed.

c. Add 20 µL of hybridization solution 1 to each hybridization tube 1 and to the first blank, using a repeating pipette.

d. Add 20 µL of hybridization solution 2 to each hybridization tube 2 using a repeating pipette.

e. Add 20 µL of hybridization solution 3 to each hybridization tube 3 using a repeating pipette.

f. Add 20 µL of hybridization solution 4 to each hybridization tube 4 using a repeating pipette.

g. For each amplified sample, add 100 µL detection diluent to a fresh test tube using a repeating pipette.

h. Close the tubes containing detection diluent.

i. For each amplified sample:

1) Open one of the test tubes containing detection diluent.
2) Add 5.0 µL amplified sample.
3) Close test tube and vortex.
4) Spin test tube.

j. For each amplified sample (diluted in step I.):

1) Add 5.0 µL diluted amplified sample to each of the four hybridization tubes, using a fresh pipette tip for each addition.

**NOTE: AVOID CONTACT OF THE PIPETTE TIP WITH THE INSIDE WALL OF THE HYBRIDIZATION TUBES. THE TIP SHOULD ONLY TOUCH THE HYBRIDIZATION SOLUTION IN THE TUBES. USE 50 mL VIALS WITH SCREW CAPS FOR DISPOSAL OF THE PIPETTE TIPS TO REDUCE CONTAMINATION RISKS**.

2) Add 5.0 µL detection diluent to each of four blanks.
3) Mix hybridization tubes and blanks until an opaque solution is formed (either mix simultaneously, e.g., using a shaker (1100 rpm), or vortex one by one).
4) Cover hybridization tubes and blanks with adhesive tape.

k. Incubate all hybridization tubes and blanks for 30 $\pm$ 1 minutes at 41 $\pm$ 0.5$^{o}$C for hybridization.

NOTE: DURING HYBRIDIZATION, MIX HYBRIDIZATION TUBES AND BLANKS EVERY 10 MINUTES.

l. Add 300 µL NASBA™ QR System Assay Buffer (refer to NASBA™ QR System Operator's Manual) to each hybridization tube and blank, using a repeating pipette. In addition, add 300 µL of instrument reference solution to a fresh 5 mL polypropylene tube.

NOTE: THE TUBES CAN NOW BE IDENTIFIED BY THE COLOR OF THEIR CONTENT (WT= COLORLESS; Qa = LIGHT BLUE; Qb = MEDIUM BLUE; Qc = DARK BLUE).

m. Perform any required NASBA™ QR System maintenance procedures (refer to Section VI.).

n. Place all hybridization tubes and blanks on the instrument carousel (for positioning, refer to Section VI.). Instrument reference solution tube should be placed in position #1 of the carousel. The Hybridization blank should be placed in position #2 of the instrument carousel.

o. Run assay (refer to Section VI. for creating, editing, and running of the job list).

## VI. INSTRUMENTATION

A. Entering System

1. Log on to the NASBA™ QR System by selecting the appropriate user name from those displayed on the screen. Enter your password and the system will allow you access to the main menu.

B. Creating Work list

1. Select "ROUTINE" from the main menu and then select the subtopic "New Run." A different screen will then be displayed.

2. Select "HIV Quantitation" from this new screen. Select which type of calibrator that was used in the assay (diluted or concentrated).

3. Enter the individual samples and after each select the “Add to List" option. Keep adding samples until all the samples are entered or you reach the end of the Work list (maximum of 12 samples/Work list).

C. Loading Machine

1. Remove the carousel from the reader by releasing the cord from the top of the carousel and then release the carousel from the reader by pushing the unlocking lever to the right.

2. Remove the cover on top of the carousel by sliding it to the side. The carousel is now ready for loading.

3. Load the carousel as follows:

| POSITION | CONTENTS |
|---|---|
| 1 | REFERENCE SOLUTION |
| 2 | ASSAY NEGATIVE CONTROL |
| 3 | WT TUBE PATIENT ONE |
| 4 | Qa TUBE PATIENT ONE |
| 5 | Qb TUBE PATIENT ONE |
| 6 | Qc TUBE PATIENT ONE |
| 7 | WT TUBE PATIENT TWO |
| 8 | Qa TUBE PATIENT TWO |
| 9 | QB TUBE PATIENT TWO |
| 10 | Qc TUBE PATIENT TWO |

4. Continue this pattern until all patient tubes have been put in the carousel.

5. After all tubes have been loaded into the carousel, place the carousel on the reader and push until a click is heard. Reconnect the cord to the top of the carousel.

D. Running Work list

1. Select "Run Work list". The carousel should spin to read position one in about 30 seconds, if it doesn't consult operating manual for explanation.

2. After last tube has been read and the machine has become idle, the results of the assay can be displayed and printed. Select "Assay results" from the menu and the results will be displayed on the screen. Select the Print option at the bottom of the screen for a hard copy of the Work list's data.

E. Saving the Run

1. Select "Save Run as" and the system will prompt you to accept the name the system has selected or give you the option to name the filename yourself.

## VII. RESULTS

The NASBA™ QR System automatically calculates the original HIV-1 RNA plasma or serum level in the volume of sample added to the lysis buffer (for viewing, printing, storing, or retrieving of results refer to Section VI.).

There are three types of results obtained from the reader: Valid, Warning, and Invalid.

A. Valid Result-A valid result can be reported normally without any further analysis.

B. Warning Result-A Warning result is one that can be reported after further analysis of the assay result or is one that may have to be repeated. A sample that gets a "warning" result can usually fall into one of two categories. The first is the Qa standard is above the threshold of the reader, this will be displayed as the Qa value of 9999999. This result is caused by exceptional amplification of both standards and patient RNA. If the patient value (WT signal) falls between the two other standards values then the result can be reported out. If the patient value (WT signal) is outside this range then the RNA detection section of the procedure must be repeated with a slight alteration in the procedure. This slight change is: increase the original dilution of the sample that was subjected to the detection process. (i.e., if the original dilution was 5 µL patient sample into 100 µL diluent (1:21), then increase dilution to 1:41 by adding 2.5 µL patient sample into 100 µL diluent).The second reason is that the standard curve that the sample is being evaluated on isn't "perfect". This problem doesn't need to be corrected if there is no WT signal (i.e., if the WT signal is 100 or less, which corresponds to an answer of "Less than Lower Limit); that answer could go out normally. If the WT signal is greater than 100, the problem

usually can be resulted by decreasing the hybridization dilution (i.e., if original dilution was 1:61 then decrease dilution to 1:10 and rehybridize and redetect).

C. An Invalid result cannot be reported under any circumstance. This result usually causes the sample to be repeated on the next run. If the result was invalid because the ratio(s) between one or several of the standards is incorrect (too low or too high), then repeat the detection part of the procedure. If upon completion of the repeat detection the sample is still invalid, the sample must undergo the complete procedure on the next run. If the signals were all "1"s then the entire procedure must be completed so a valid answer can be obtained for the patient.

D. If a sample is repeated and still produces an "Invalid" result, then the physician will be notified and a free text message will be entered with the worksheet.

## VIII. INTERPRETATION OF RESULTS

Present data are insufficient to establish a direct correlation between HIV-1 RNA levels and the clinical outcome of the disease. However, studies reveal that HIV-1 RNA levels are associated with disease stage and CD4+ T cell counts. In addition, levels change rapidly in response to effective therapy.

With primary infection, HIV-1 RNA levels reach a peak (coinciding with seroconversion) within days or weeks after onset of symptoms and decline rapidly thereafter. Primary infection is followed by an asymptomatic stage and later by persistent lymphadenopathy, where levels remain relatively low. With progression to AIDS-related complex, levels increase significantly. Another significant rise in levels comparable to those in primary infection is observed after progression to AIDS.

Throughout disease progression, a rise in HIV-1 RNA levels is significantly correlated to a decline in CD4+ T cell count.

High individual peak levels in primary infection and persisting high levels during the following stages may imply a negative prognosis.

In a subgroup of patients, treatment with zidovudine (AZT) is followed by a rapid decrease in HIV-1 RNA levels. A rapid rebound to pretreatment levels is observed after discontinuation of therapy, suggesting continuing viral replication throughout treatment. Other patients respond only transiently to therapy or do not respond at all. Quantitation of HIV-1 RNA levels can help to identify non-responders and spare them the consequences of ineffective AZT therapy.

## IX. PROCEDURAL NOTES

Perform nucleic acid release, isolation, amplification and detection in separate laboratory areas.

Air from the detection area must not be allowed to enter the other areas (detection should be performed in a fume hood).

Keep all tubes and vials closed when not in use.

Do not use pipettes and other equipment which have been used in one laboratory area in the other areas.

Use a fresh pipette or pipette tip for each pipetting action.

Use pipettes with aerosol resistant tips or air displacement pipettes for fluids possibly containing nucleic acid.

Pipetting solutions always must be performed out of or into an isolated tube that is opened and closed exclusively for this action. All other tubes and vials should be kept closed and separated from the one handled.

Use disposable gloves when working with clinical material possibly containing HIV-1 or amplified material. If possible, change gloves after each pipetting step in the test procedure, especially after contact with possibly contaminated material.

In the amplification lab area, the use of water baths instead of heating blocks increases the risk of contamination.

Soak tube racks used during nucleic acid isolation in a detergent (EXTRAN-1000) for at least one hour after each test run.

All reagents and specimens must be mixed thoroughly before use. NOTE: ENZYMES MUST NOT BE VORTEXED. MIX GENTLY, E.G., BY TAPPING THE TUBE.

## X. LIMITATIONS OF THE PROCEDURE

A negative test result does not exclude the possibility of exposure to, or infection with, HIV-1.

The assay must be performed in strict accordance with the instructions in this package insert and the NASBA™ QR System Operator's Manual to obtain accurate, reproducible results.

Samples from individuals infected with HIV-2 may exhibit cross-reactivity.

In addition to quantitative HIV-1 RNA load, other virologic or immunologic factors may contribute to variable rates of CD4+ T cell counts and clinical outcome of the disease.

## XI. REFERENCES

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